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Relationship between peritubular oncotic pressure gradients and morphology in isolated proximal tubules

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Relationship between peritubular oncotic pressure gradients and morphology in isolated proximal tubules. Previous studies have demonstrated that net sodium and water flux across the isolated perfused proximal convoluted tubule of the rabbit can be increased by elevating the protein concentration of the bath and at least part of the protein effect is mediated through oncotic forces. This study was designed to determine the pathway of the reabsorbed fluid by examining the morphologic characteristics of the epithelium as the ambient protein concentration was varied. Isolated segments of rabbit proximal convoluted tubule were preserved *in vitro* for light and electron microscopy by intraluminal perfusion of an isotonic fixative solution as the tubule was bathed in a hyperoncotic, an iso-oncotic or a hypo-oncotic protein-free ultrafiltrate of serum. Widening of lateral and basilar intercellular spaces was observed in proximal convoluted tubules exposed to hyperoncotic and iso-oncotic protein bathing solutions at the time of fixation. The widened intercellular spaces contained protein whose presence was confirmed with electron microscopic autoradiography using ^{125}I-labeled albumin as a marker. These studies demonstrate an association between the width of the intercellular space and net sodium and water flux in the mammalian proximal convoluted tubule. The results suggest the possibility that the widened lateral and basilar intercellular spaces may represent a component of at least one pathway by way of which fluid is reabsorbed across the epithelium of the proximal convoluted tubule *in vitro* in response to peritubular oncotic pressure gradients. In addition, since albumin is capable of crossing the basement membrane of the tubule, the oncotic effect of protein in this model is probably not mediated solely across the basement membrane, but may effect an osmotic driving force across either the tight junction or the lateral plasma membrane of the cell or both in the isolated proximal tubule.

Relation entre les gradients de pression oncotique péritubulaire et la morphologie du tube proximal isolé. Des travaux antérieurs ont montré que le flux net d'eau et de sodium à travers le tube contourné proximal de lapin isolé et perfusé peut être accru par l'augmentation de la concentratione protéin des dans le bain et qu'au moins une partie de l'effet des protéines est exercé par l'intermédiaire des forces oncotiques. Ce protocole a pour but la détermination des voies de réabsorption du liquide par l'étude de la morphologie de l'épithélium alors que la de tube contourné proximal de lapin ont été préparés *in vitro* concentration ambiante de protéines varie. Des segments isolés aux fins d'examen en microscopie photonique et électronique par la perfusion intra luminale d'une solution de fixative isotonique alors que le tubule baignait dans un ultrafiltrat de sérum hyperoncotique, isooncotique, ou encore hypooncotique exempt de protéines. L'élargissement des espaces intercellulaires latéraux et basilaires est observé dans les tubes contournés proximaux exposés à des solutions hyperoncotiques ou isooncotique au moment de la fixation. Les espaces intercellulaires élargis contiennent des protéines dont la présence est confirmée par des autoradiographies utilisant l'albumine ^{125}I comme marqueur. Ces résultats démontrent une relation entre la largeur de l'espace intercellulaire et le flux net d'eau et de sodium dans le tube contourné proximal des mammifères. Ils suggèrent que les espaces intercellulaires basilaires et latéraux élargis puissent représenter un élément d'au moins une voie par laquelle le liquide est réabsorbé *in vitro* à travers l'épithélium du tube contourné proximal en fonction des gradients péritubulaires de pression oncotique. De plus, du fait que l'albumine peut traverser la membrane basale du tubule, l'effet oncotique des protéines dans ce modèle n'est probablement pas exercé seulement à travers la membrane basale mais peut aussi réaliser une force osmotique à travers la jonction serrée ou la membrane plasmique latérale de la cellule (ou les deux) du tube proximal isolé.

The presence of protein on the antiluminal surface of the proximal tubule can enhance net sodium and water reabsorption. In the isolated perfused proximal convoluted tubule (PCT) of the rabbit, Imai and Kokko [1] demonstrated that the magnitude of net sodium and water reabsorption could be directly related to the concentration of protein in the bath. The transepithelial potential difference was not affected by lowering or raising the concentration of the bath protein, but ^{14}C-sucrose permeability, thought to reflect paracellular ion movement, was increased when the peritubular protein concentration was decreased. From these initial studies it was concluded that the peritubular protein acted primarily by altering the rate of backleak of reabsorbate through extracellular pathways. These initial findings have been confirmed by Grantham, Qualizza and Welling in the isolated

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tubule [2]. Recent studies by Imai and Kokko [3] have demonstrated that at least part of the protein effect is mediated via oncotic forces. In addition, both *in vivo* [4] and *in vitro* [3] evidence suggests that active transport pathways are "coupled" to pathways that are responsive to oncotic forces.

The present investigation was initiated to determine the intracellular and extracellular pathway(s) taken by fluid reabsorbed across the epithelium of the isolated PCT of the rabbit, by examining the morphology of the tubule during different rates of net sodium and water reabsorption occurring in response to peritubular oncotic pressure gradients.

Methods

Isolated segments of PCT were obtained from young healthy female white New Zealand rabbits and prepared for microperfusion using techniques previously described [5, 6]. The rabbits were killed by guillotine and a 1-mm slice of kidney was excised and quickly transferred to a chilled dish of rabbit serum where the individual segments of PCT were dissected free. The tubules were then transferred by pipette to a perfusion chamber containing rabbit serum which was maintained at 37°C and at pH 7.4, the latter by continuous bubbling with 95% O_2–5% CO_2 gas mixture. After the initial hook-up, the tubule was continuously perfused with an isosmolal ultrafiltrate of commercially available rabbit serum. Three baths were used in the experimental protocol: *a*) commercially available regular rabbit serum (iso-oncotic bath), *b*) ultrafiltrate of rabbit serum (hypo-oncotic bath) and *c*) serum remnant of ultrafiltration (hyperoncotic bath). The exact composition of these bathing solutions and the method of preparation have been published previously [1].

Each tubule was allowed to stabilize for 20 to 30 min before initiation of the experimental protocol. During this time, the tubule was perfused with an iso-osmotic protein-free ultrafiltrate of the same rabbit serum as used for the bath. The bath was then changed to vary its oncotic pressure. After the change of bath, the tubule was permitted to reach a new steady state condition during the next 20 to 30 min. At this point, the perfusion solution was quickly exchanged for a fixative solution and the tubule was preserved for light and electron microscopy by intraluminal perfusion of an iso-osmotic 2% glutaraldehyde solution buffered in 0.05 M sodium cacodylate at pH 7.2 to 7.6, containing 0.5% lissamine green as a flow marker. Preliminary investigations employing hypertonic and hypotonic aldehyde and hypotonic osmium tetroxide fixatives demonstrated that the morphologic results were independent of the osmolality of the fixative solution when the tubule was fixed from the luminal surface. Fixation of the tubule from the luminal surface was utilized in all experiments in order to avoid precipitation of the protein in the bath, which could alter the oncotic pressure of the bath before adequate cellular preservation was accomplished. The period of perfusion fixation was continued for 15 to 20 min, after which the individual tubules were removed from the perfusion pipettes and transferred to small plastic vials (Beem capsule, LKB Instruments, Inc., Rockville, MD) that contained the same fixative solution. The tubules were then fixed for an additional two to four hours before undergoing an overnight buffer rinse in 0.15 M sodium cacodylate. In some instances, the tubules were held in the buffer solution for several days at 0°C before subsequent tissue processing. The individual tubules were postfixed in 1 or 2% osmium tetroxide in *s*-collidine buffer for one hour at 4°C and then dehydrated in a graded series of alcohol solutions before being embedded in epoxy resin (Epon) [7]. Some tubules were subjected to en bloc staining for one hour at room temperature with 0.5% aqueous uranyl acetate in *s*-collidine buffer immediately after postfixation and before alcohol dehydration. Sections 1 μ in thickness were stained with toluidine blue. Thin sections for electron microscopy were cut with diamond knives and doubly stained with aqueous uranyl acetate [8] and lead citrate [9] or left unstained before examination and photographic recording in an electron microscope (AEI 6B) Thirty-two individual tubules were studied in the initial phase of the investigation.

The identity of the electron-dense material in the intercellular spaces of tubules bathed in protein was next established using electron microscopic autoradiography. A group of five tubules was exposed to either a hyperoncotic (two) or iso-oncotic (three) protein bath that contained ^{125}I-labeled albumin. The tubules underwent perfusion fixation and tissue processing in a manner identical to that of all other tubules. The autoradiographic techniques that were employed were similar to those previously described by Salpeter, Bachmann and Salpeter [10] and by Salpeter and Bachmann [11]. Ribbons of thin sections cut at 1000 ± 100 Å were transferred to a glass microscope slide previously coated with 0.5% collodion. The sections were then stained for two hours at room temperature with 2% uranyl acetate. Thereafter, the sections were vacuum-coated with a 50-Å layer of carbon. The slides holding the sections were then dipped in a beaker containing emulsion (Ilford L4) at 50°C and air dried in a vertical position. Slides were stored at 2°C for a period of exposure ranging from

two weeks to three months. The sections were developed for three minutes at 23 to 24°C in Microdol X (Eastman Kodak, Co., Rochester, NY) and fixed for one minute at room temperature. The specimen "sandwich" was then stripped onto water and 200 mesh copper grids were placed over the sections. The grids with the sections were removed from the surface of the water by suction. A total of 518 micrographs was taken of the autoradiographic specimens at a constant magnification of ×10,000. They were enlarged photographically to a final magnification of ×26,500 before analysis.

Grain distribution and grain density (developed silver grains/unit area) were determined in the three tubules bathed in iso-oncotic protein. The two tubules bathed in hyperoncotic protein containing ^{125}I-labeled albumin were used for qualitative evaluation only. The analysis of grain distribution within the three tubules bathed in the iso-oncotic protein was performed in a manner designed to test the hypothesis that the source of the silver grains was ^{125}I-labeled albumin within the intercellular space. For this analysis and for the subsequent calculations, a half-distance (HD)[1] of 1500 Å was used and the intercellular space was treated as a solid band according to the method described by Salpeter, Bachmann and Salpeter [10]. First, the distance was measured from the center of each developed grain (ie., the center of the smallest circle enclosing the entire grain) to the center of the closest intercellular space. From these data a histogram was constructed showing the number of grain midpoints per unit of perpendicular distance from the nearest intercellular space. Next, to establish the grain density distribution, a grid with uniformly spaced points was placed over the same photographs, and the perpendicular distance from each point to the nearest intercellular space was measured and tabulated. A second histogram was then constructed depicting the grain density by dividing the number of grains per unit of distance by the number of points in the same unit of distance. This density histogram was then compared with normalized universal density curves (obtained from reference 10), and the validity of the original hypothesis was tested by comparing the experimentally derived curve with the theoretical curve. In the analysis of the grain distribution, no grains were counted over the epithelium that fell within 2.0 cm (after photographic enlargement) or 5 HD of the inner edge of the basement membrane of the tubule. This restriction was designed to avoid inclusion of grains resulting from the presence of ^{125}I-labeled albumin in the basement membrane of the tubule or fixed to the outer surface of the basement membrane.

High specific activity ^{125}I-bovine albumin was prepared for the autoradiographic studies according to the method of Hunter and Greenwood [12]. Using chloramine-T as the oxidizing agent, 0.025 ml of concentrated bovine albumin (35 g/100 ml) in 0.025 ml of 0.5 M phosphate buffer was reacted with 10 mCi of ^{125}I-sodium. The labeled albumin was purified by passage through a Sephadex G-25 column from which it was eluted with rabbit serum. The final concentration of ^{125}I radioactivity in 1 ml of rabbit serum was 2.3 mCi. The specific activity of the albumin prepared in this manner was 36 μCi/mg. Immediately prior to use, the ^{125}I-labeled albumin was dialyzed in a cellophane bag against Ringer's solution (300 mOsm/kg H_2O) to remove any small mol wt fragments which might have resulted from autodestruction. One millicurie of labeled albumin was added to the bath.

Quantitative morphometric techniques were employed to determine the difference between the width of the lateral and basilar intercellular spaces in the

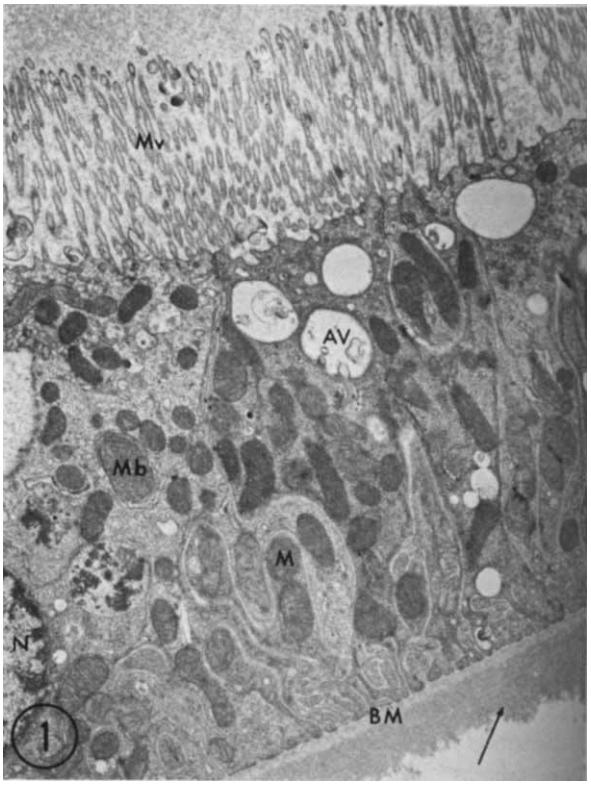

Fig. 1. *Low power electron micrograph of a rabbit proximal convoluted tubule (PCT) fixed by intraluminal perfusion with a 2% glutaraldehyde solution while immersed in an iso-oncotic protein bath.* Protein (*arrow*) has become fixed to the outer surface of the basement membrane (BM) during tissue preservation. N, nucleus; AV, apical vacuole; M, mitochondrion; Mb, microbody; Mv, microvilli; magnification, ×7,500.

[1] The HD is defined as the distance from a line source of radioactivity within which 50% of the total developed grains fall.

Fig. 2. *Electron micrograph depicting the irregular configuration of the intercellular spaces* (arrows) *of a PCT bathed in a hypo-oncotic protein-free solution at the time of fixation.* Note that the spaces are electron-lucent. M, mitochondrion; BM, basement membrane; Mb, microbody; magnification, ×47,000.

tubules bathed in iso-oncotic and hyperoncotic protein solutions and those bathed in the protein-free hypo-oncotic solution. Eight electron micrographs were taken of each tubule at a magnification of ×20,000. A total of ten measurements was made directly from each negative. The measurements were confined to the lower one-third of the cell and were taken between the outer leaflets of the two opposing plasma membranes of adjacent cells where the cell membranes ran parallel with one another. Twenty-six tubules were studied in this manner. The electron microscope was calibrated using a carbon replica of a 28,800-line block to establish the actual magnification.

Numerical data were analyzed by Student's *t* test. Data are expressed as mean ±SD, and *P* values less than 0.05 are considered to be significant.

Fig. 3. *Electron micrograph illustrating the uniform widening of the intercellular space* (arrows) *in the basal region of a PCT bathed in an iso-oncotic protein solution at the time of fixation.* Note the electron-dense protein fixed to the outer surface of the basement membrane (BM) and the presence of similar appearing material in the intercellular spaces. MvB, multivesicular body; magnification, ×47,000.

Results

As demonstrated in Fig. 1, the overall cellular preservation of proximal tubules fixed by intraluminal perfusion was quite adequate and compared favorably with results obtained using other techniques of fixation that are more commonly employed in the kidney such as *in vivo* drip-fixation [13], *in vivo* intravascular perfusion fixation [14] and *in vivo* intraluminal perfusion fixation [15]. The organization and appearance of the various subcellular organelles was nearly identical to that described in isolated proximal tubules of the rabbit preserved by the addition of fixative to the bath [16].

Effect of peritubular oncotic pressure gradients on

Table 1. Summary of the effects of varying the oncotic pressure on intercellular space width in isolated perfused proximal tubules[a]

Condition	Tubules, *N*	Measurements, *N*	Width of intercellular spacing, Å	
Hypo-oncotic bath	10	800	260±38	
Iso-oncotic bath	9	720	333±36	$P<0.001$[b]
Hyperoncotic bath	7	560	367±15	$P<0.001$[b] $P<0.05$[c]

[a] Numbers are given as mean ±SD.
[b] Compared to hypo-oncotic bath.
[c] Compared to iso-oncotic bath.

Fig. 4. *Electron micrograph demonstrating the presence of protein (albumin) in the uniformly widened lateral intercellular space* (arrows) *extending to the level of the junctional complex (JC) in the apical region of the cell.* This PCT was bathed in an iso-oncotic protein solution at the time of fixation. L, lysosome; M, mitochondrion; magnification, ×30,000.

intercellular space configuration. Fig. 2 demonstrates the typical appearance of the basal two-thirds of the epithelium lining a PCT bathed in a protein-free hypo-oncotic solution at the time of fixation. The configuration of the lateral and basilar intercellular space was extremely irregular and the space itself was electron-lucent. The mean width of the intercellular space measured in ten tubules bathed in the hypo-oncotic solution was 260 ± 38 SD Å ($N=800$) (Table 1). Fig. 3 illustrates the typical appearance of a proximal tubule bathed in an iso-oncotic protein solution at the time of fixation. There was uniform widening of the intercellular space, which extended from the base of the cell to the tight junction or *zonula occludens* (Fig. 4). In addition to their generally regular configuration, the intercellular spaces contained an electron-dense material similar in appearance to the layer of protein that was often fixed to the outside surface of the basement membrane. In some instances, localized dilatations of the intercellular space were encountered in addition to the uniform widening. As shown in Table 1, the mean width of the intercellular space measured in nine tubules bathed in the iso-oncotic protein solution was 333 ± 36 SD Å ($N=720$). Proximal tubules bathed in a hyperoncotic protein solution exhibited the same uniform widening of intercellular spaces and the electron-dense intercellular material. The mean width of the intercellular space measured in seven tubules was 367 ± 15 SD Å ($N=560$) (Table 1). The difference in intercellular space width between tubules bathed in the hypo-oncotic protein-free bath and the iso-oncotic protein bath was highly significant ($P<0.001$), as was the difference in intercellular space width between tubules bathed in hypo-oncotic protein-free and hyper-oncotic protein solutions ($P<0.001$). There was also a significant difference in the width of the intercellular spaces in those tubules bathed in the iso-oncotic vs. the hyperoncotic solutions ($P<0.05$).

The autoradiographic studies revealed that the intercellular spaces of those tubules bathed in protein contained ^{125}I-labeled albumin which presumably had crossed the basement membrane from the bath (Figs. 5 and 6). Numerous grains were located over the basement membrane and the layer of protein fixed to the outer surface of the basement membrane. In areas of localized dilatation of the intercellular space, the presence of ^{125}I-labeled albumin was particularly evident (Fig. 7). Results of the analysis of grain distribution over the proximal tubule epithelium before and after correction for density distribution and background are shown in Figs. 8 and 9. The findings confirm the qualitative analysis (Figs. 5 through 7) and support the hypothesis that the source of the radioactivity is iodinated albumin in the intercellular spaces. Fig. 8 demonstrates that more than 72% of the grains fell within one HD of the nearest intercellular space. When the density distribution was then calculated to

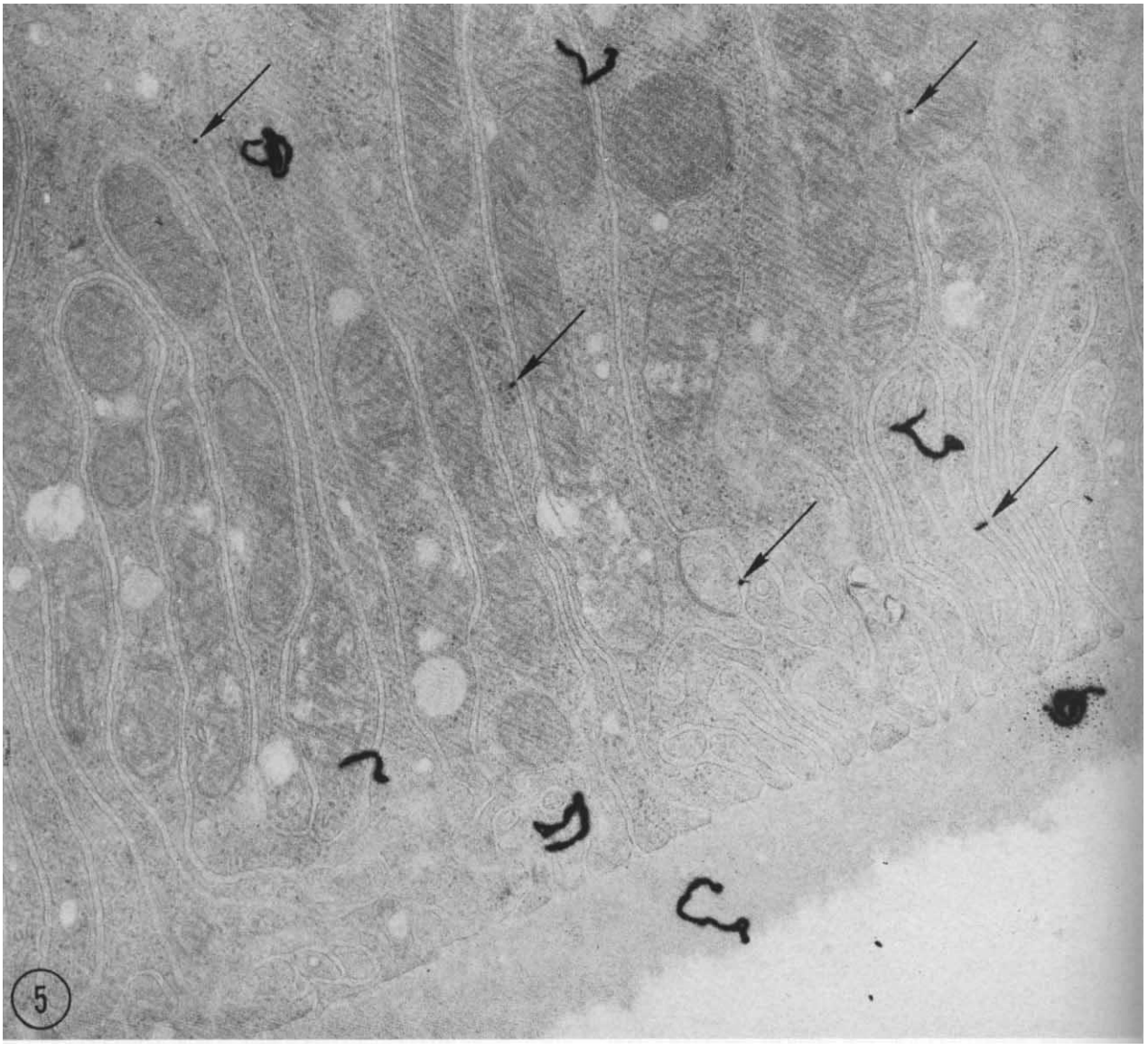

Fig. 5. *Electron microscopic autoradiograph of a PCT fixed while bathed in an iso-oncotic bath which contained* ^{125}*I-labeled albumin.* Developed silver grains overlie the intercellular spaces, the basement membrane and the protein layer fixed to the outer surface of the basement membrane. *Arrows* denote additional grains which are less fully developed. Magnification, × 34,500.

correct for the irregular distribution of the intercellular space in the tubule and the results compared with the theoretical curve derived for a solid band source of radioactivity (comparable to the intercellular space), a close fit of the theoretical and experimental curves was obtained (Fig. 9).

Discussion

The presence of widened intercellular spaces in those tubules exposed to a peritubular oncotic pressure gradient relates well to previously measured rates of net sodium and water reabsorption under the same conditions utilized in the present set of experiments. Imai and Kokko [1], studying the same model, observed a net water flux of 0.51 ± 0.09 SEM nl mm^{-1} min^{-1} when the bath protein concentration was reduced to zero (hypo-oncotic), a 48% increase to 0.99 ± 0.10 SEM nl mm^{-1} min^{-1} with a bath protein concentration of 6.4 g/100 ml (iso-oncotic) and an additional 28% increase in net water flux to 1.38 ± 0.15 SEM nl mm^{-1} min^{-1} when the bath protein concentration was 12.5 g/100 ml (hyperoncotic). In the present study, the mean width of the intercellular space was 22% greater in tubules bathed in an iso-oncotic protein solution at the time of fixation as

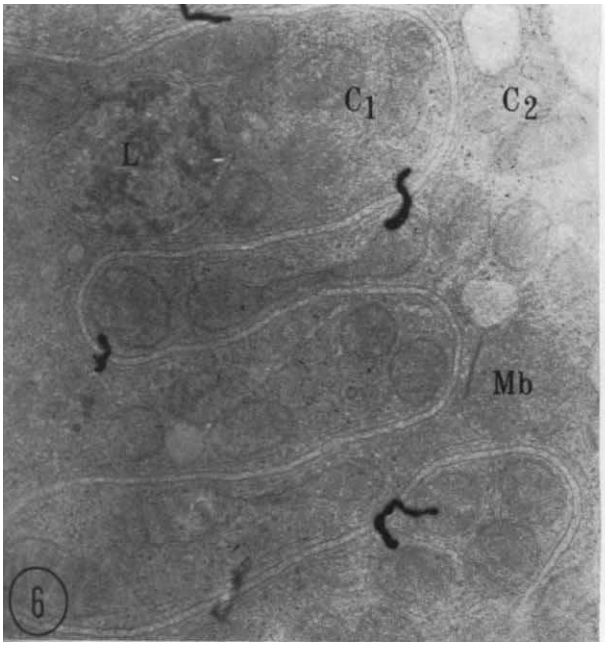

Fig. 6. *Electron microscopic autoradiograph from another region of the same PCT depicted in Fig. 5.* Several silver grains overlie the uniformly widened lateral intercellular space separating two adjacent epithelial cells (C_1 and C_2). Mb, microbody; L, lysosome; magnification, × 19,500.

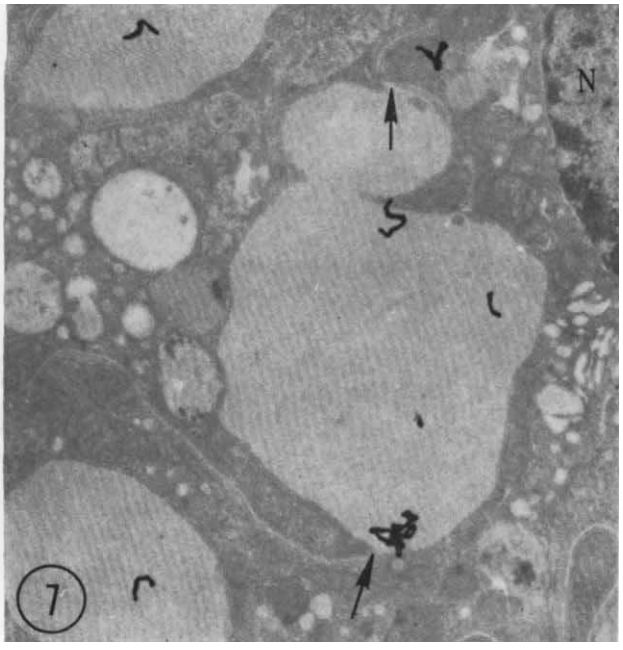

Fig. 7. *Electron microscopic autoradiograph from a PCT fixed while bathed in a hyperoncotic protein solution containing ^{125}I-labeled albumin.* Segments of widely dilated lateral intercellular space contain several developed silver grains. *Arrows* denote the points where the uniform widening of the intercellular space gives way to the marked dilatation. N, nucleus; magnification, × 12,500.

compared to tubules bathed in a protein-free solution at fixation. There was another 10% increase in mean width of the intercellular space in tubules bathed in a hyperoncotic protein solution. The combined structural-functional observations suggest the possibility that in the isolated perfused PCT the widened intercellular spaces represent at least a partial pathway through which fluid and solutes traverse the epithelium from lumen to bath in response to oncotic pressure gradients. The present findings differ from those of Burg and Grantham [17], who were unable to detect differences between the ultrastructure of intercellular spaces in transporting vs. nontransporting isolated PCT's. However, the experimental conditions under which the ultrastructure of the tubule was examined by these workers were considerably different from those of the present study, making it quite difficult to make a valid comparison of the results obtained in the two studies. It was observed that the width of the lateral intercellular spaces was on the order of 200 Å in a PCT that was actively pumping fluid at a rate of 0.83 nl/mm/min just prior to fixation [17]. Although the composition of the fluid bathing the tubule was not given by the authors, it is interesting that the value for the measured width of the intercellular space corresponded rather closely to similar measurements obtained in the present study for intercellular space width (260 ± 38 Å) in PCT's bathed in a hypo-oncotic protein-free solution. Burg and Grantham [17] did not evaluate the width of lateral intercellular spaces of PCT's bathed in protein solutions in which net fluid reabsorption was increased.

The exact role of protein in the net reabsorption of ions and water in the proximal tubule has been the subject of considerable investigation. Although the difference in transepithelial colloid osmotic pressure across the tubule may play a major role in the reabsorption of sodium and water as suggested by many workers (see reference 4 for detailed discussion), the magnitude of such a driving force that would be required coupled with the rather low hydraulic permeability of the proximal tubule epithelium [6, 18, 19] suggests that the bulk of fluid reabsorption in the proximal tubule does not occur via this mechanism. To surmount this problem, Lewy and Windhager [20] proposed that the oncotic effect of protein in the peritubular capillaries was chiefly directed at the removal of reabsorbate that was already present in the intercellular space as the result of active ion transport. Such a mechanism requires active transport processes to be intact to permit the modifying influence of the peritubular protein to occur. Recent strong support for

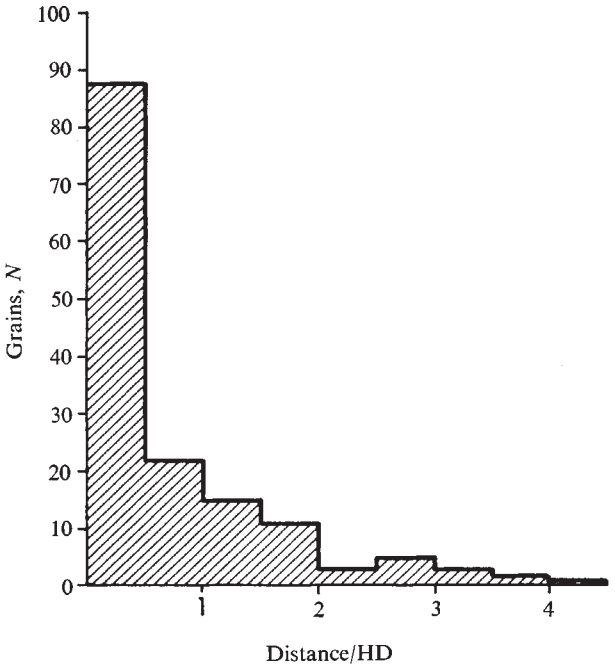

Fig. 8. *Histogram depicting the number of grain midpoints per unit of perpendicular distance from the center of the uniformly widened intercellular spaces of the three tubules studied by autoradiography that were bathed in an iso-oncotic protein bath to which ^{125}I-labeled albumin was added.* Number of grains is shown on the ordinate. Distance steps of 750 Å representing one-half of a half-distance (HD) (see text for definition) are shown on the abscissa. Seventy-two percent of the grains fell within 1500 Å or one HD of the center of the nearest intercellular space.

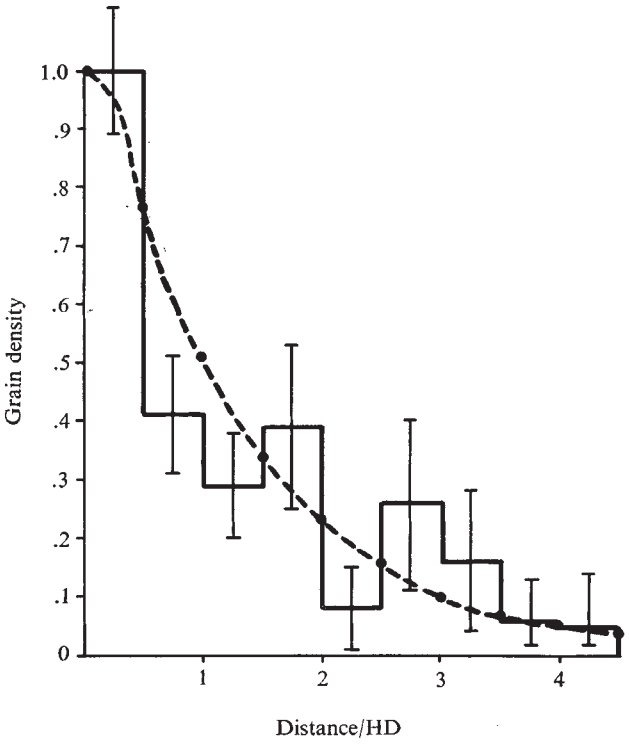

Fig. 9. *Histogram of the grain density obtained by dividing the number of silver grains in each perpendicular distance (Fig. 8) by the number of density points for the same perpendicular distance (see text for explanation).* This provides a density distribution of developed grains which corrects for the irregular shape and distribution of the intercellular spaces in the tubule. The *dotted line* represents a superimposed theoretical curve derived from the work of Salpeter, Bachmann and Salpeter [10], which would be expected for a solid line source of ^{125}I under the conditions of the present experiment.

this formulation has been provided by the studies of Green, Windhager and Giebisch in the intact rat kidney [4] and Imai and Kokko employing the isolated perfused rabbit tubule [3]. Both groups of investigators observed that albumin applied intraluminally did not affect net fluid reabsorption by the proximal tubule, contrary to a recent report [21], while inhibition of active sodium transport largely negated the influence of peritubular albumin on fluid reabsorption. It is also worth noting the similarities in reabsorptive properties that appear to exist under the above experimental conditions in the *in vivo* and *in vitro* models despite the absence of an interstitium and a peritubular capillary wall interposed between the basement membrane of the tubule and the albumin in the isolated tubule preparation.

The present observations suggest that in the intact isolated rabbit proximal tubule, the oncotic effect of protein may not be exerted solely across the basement membrane as first suspected [1]. The initial electron microscopic observations demonstrating an electron-dense material within the intercellular space of tubules exposed to protein suggested that albumin had crossed the basement membrane from the bath (Figs. 3 and 4).

The suspected presence of albumin in the intercellular space was then confirmed by the autoradiographic studies employing ^{125}I-labeled albumin. The presence of albumin in the intercellular space is entirely consistent with results of certain earlier investigations employing various tracer techniques. There is ample experimental evidence that proteins with a mol wt less than that of albumin are capable of penetrating the basement membrane of the proximal tubule and entering the intercellular space from the peritubular surface. Bentzel et al [22] observed that horseradish peroxidase with a mol wt of approximately 40,000 easily crossed the basement membrane of proximal tubules of intact doubly perfused *Necturus* kidneys. Recently, Ottosen and Maunsbach [23] observed the same phenomenon with horseradish peroxidase in proximal tubules of the kidney in the flounder, *Pleuronectes platessa*. Of more direct importance, Welling and Grantham [24] have demonstrated with tracer techniques, using ^{131}I-labeled albumin, that the isolated basement

membranes of rabbit proximal convoluted tubules obtained by exposure to sodium desoxycholate or osmotic shock were moderately permeable to albumin. Thus, the results of the present study provide additional evidence that the basement membranes of proximal tubules are moderately permeable to albumin, at least *in vitro*, and also tend to exclude the possibility that physical damage accounted for the albumin permeability in the isolated tubular basement membranes reported by Welling and Grantham [24]. In the intact isolated perfused renal tubule, it is quite possible that the albumin which crosses the basement membrane exerts an oncotic or osmotic effect across the tight junction or the lateral plasma membrane of the cell or both. The results of the present study do not permit further delineation of the possible site of action of that protein within the intercellular space. The results, however, would appear to be entirely consistent with the concept that the oncotic effect of protein facilitates net reabsorption of sodium and water by decreasing backleak of resorbate from the intercellular space through the tight junction into the tubule lumen as proposed by Lewy and Windhager [20] and Green, Windhager and Giebisch [4]. Welling and Grantham [24] noted that while the isolated basement membrane was moderately permeable to albumin, a significant osmotic gradient was still maintained across the basement membrane.

Whether the basement membrane of the proximal tubule in the intact kidney is also permeable to albumin and whether any functional significance can be attached to such permeability characteristics *in vivo* remain unanswered questions. Indirect evidence suggests the likelihood, however, that albumin can gain access to the lateral intercellular space of proximal tubules *in vivo*. Under normal circumstances, protein leaks out of the peritubular capillaries into the interstitium. In the dog the concentration of protein in the renal lymph which probably reflects the protein concentration in the interstitial fluid bathing the tubules approaches 70% of that in the plasma [25, 26]. Thus, albumin is seemingly readily available to move across the basement into the intercellular space *in vivo*.

In summary, the results of the present study demonstrate an association between the width of the intercellular space and net sodium and water flux in the PCT. The findings suggest the possibility that the widened lateral and basilar intercellular spaces represent a component of at least one pathway of fluid and ion movement across the isolated proximal tubule. The studies also demonstrate that albumin, present in the bath, is capable of crossing the basement membrane of the intact isolated proximal tubule to enter the intercellular space where it may exert at least part of its oncotic or osmotic effect across the tight junction or the lateral plasma membrane of the cell or both.

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